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The relationship of serum copper and zinc levels with oxidative stress markers and other laboratory parameters in COVID-19 patients

Serum copper and zinc levels with coronavirus disease 2019

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Abstract
Aim: Serum Copper (Cu) and Zinc (Zn) levels can be associated with novel coronavirus disease 2019 (COVID-19). However, the correlation of serum Cu and Zn levels with biochemistry, hormones, and coagulation parameters has not been fully revealed. This study aims to determine serum Cu and Zn levels and their relationships with other laboratory parameters in the acute phase of COVID-19.
Material and Methods: This retrospective observational study was conducted with patients who were diagnosed with COVID-19 in a tertiary hospital. The study was continued with the remaining 116 people: 53 healthy and 63 SARS-CoV-2-positives seriously ill. All laboratory data were retrospectively scanned from patient files at the hospital information system.
Results: It was found that serum Cu, G6PD and TAS levels decreased, Zn TOS and OSI levels increased when COVID-19 patients were compared with healthy individuals. There is a positive correlation between serum Cu level and AST in COVID-19 patients, and a negative correlation between total bilirubin and LDH. There is a negative correlation between serum Zn levels and direct bilirubin, CRP, and procalcitonin.
Discussion: Many studies have been reported showing that both Cu and Zn have antiviral effects against COVID-19. Although our data support these studies, it has been revealed that serum Cu and Zn levels were correlated with AST, direct/total bilirubin, LDH, CRP, and prolactin.

Keywords
COVID-19, Copper, Zinc, G6PD, OSI

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Introduction

The current coronavirus disease-2019 (COVID-19) pandemic is due to the new coronavirus SARS-CoV-2. COVID-19 is a respiratory disease caused by a novel enveloped, positive-sense, single-stranded RNA betacoronavirus, denoted as SARS-CoV-2 [1]. Replication of the viral genome within infected cells is a key stage of the SARS-CoV-2 life cycle. It is a complex process involving the action of several viral and host proteins to perform RNA polymerization, proofreading, and final capping [1]. Once inside the cell, the infected RNA acts as a messenger RNA (mRNA), which is then translated by the host's ribosomes to produce the viral replicative enzymes, which generate new RNA genomes and mRNAs for the synthesis of the components necessary to assemble the new viral particle [1].

Data gleaned from animal and clinical studies have highlighted the prominent roles of Zn and Cu in various biological processes as a cofactor, signaling molecule, and structural element. These essential trace elements are a constituent of more than 300 metalloenzymes that participate in several cellular and metabolic processes, such as cell proliferation, differentiation, stabilization of cell membranes, redox signaling, apoptosis, RNA/DNA synthesis, and metabolism of micro-and macronutrients [2].

Zn is known to exhibit a variety of direct and indirect antiviral properties. Previous literature has demonstrated that Zn homeostasis is interconnected with the emergence of infections related to coronaviridae [3]. Zn displays antiviral properties by several physical processes, including virus attachment, penetration, infection, uncoating, and replication [4].

Cu exhibits potent virucidal properties and is thus known to neutralize a wide range of infectious viruses, such as bronchitis virus, poliovirus, influenza virus, HIV type 1, and other enveloped or nonenveloped, single- or double-stranded DNA and RNA viruses [5]. An in vivo study showed that Cu ions block the activity of papain-like protease-2, which is essential for the process of SARS-CoV-1 replication [6].

Studies have shown that Zn and Cu are components of many viral enzymes, proteases and polymerases, and that these elements are important in preventing systemic cell homeostasis and viral infection. In addition, Zn and Cu are involved in the regulation of cellular oxidative stress. The aim of this study is to compare serum zinc and copper levels with parameters that are indicative of prognosis in COVID-19 patients. In addition, it was aimed to determine the relationship of these elements with oxidative stress.

Material and Methods

This study was carried out on adults with COVID-19 admitted to Şanlıurfa Mehmet Akif İnan Research and Education Hospital, Turkey, from January to February 2020. For the diagnosis of SARS CoV-2 infection, the real-time polymerase chain reaction (RT-PCR) test of the nasopharyngeal and oropharyngeal samples was evaluated. The group consisted of SARS-CoV-2 positive severe patients hospitalized in the intensive care unit. All patients have <92 SaO2. The control group consisted of 50 healthy adults. This study has been approved by the Ethics Committee of Mehmet Akif İnan Education and Research Hospital and the Turkish Ministry of Health (E1-20–1009).

The RT-PCR results and biochemistry data of all patients treated in any clinic in our hospital who tested positive for COVID-19 were obtained from the hospital system. Their biochemistry parameters (glucose, urea, creatine, ALT, AST, total bilirubin (T-bil), direct bilirubin (D-bil), GGT, Na, K, and CRP), hormone parameters (procalcitonin, ferritin, CK-MB, and troponin), coagulation parameters (fibrinogen), procalcitonin, and D-dimer levels were studied. Biochemistry-Hormone-D-Dimer analysis, Roche Cobas 8000. Coagulation was measured automatically with Sysmex cs2500 devices (Sysmex Inc., Japan). Cu and Zn in serum were determined by flame atomic absorption spectrometry (FAAS; Perkin Elmer AAnalyst 400, USA). Cu and Zn were measured at 324.8 nm and 213.9 nm, respectively.

Total oxidant status (TOS) and total antioxidant status (TAS) were measured using Erel's methods [7]. The ratio of TOS level to TAS level was accepted as the oxidative stress index (OSI). The OSI value was calculated according to the following formula [8]. OSI (Arbitrary Unit)=TOS (μmol H2O2 Equiv/L)/TAC (mmol Trolox Equiv/L).

Statistics

The evaluation of the data was performed with the SPSS 21.00 program. Descriptive statistics were used. Mean, Standard deviation and percentages are given as descriptive statistics. Conformity of the variables to the normal distribution was examined using the visual Shapiro-Wilk test. Numerical variables, determined according to the state of normal distribution were evaluated using the independent sample T-test between the two groups. Spearman's Correlation analysis was applied to determine the relationship between numerical variables. Correlation coefficient was accepted as 0.05-0.30 low, 0.30-0.40 low-moderate, 0.40-0.60 moderate, 0.60-0.70 good, 0.70-0.75 very good, 0.75-1.00 excellent correlation. In the statistical analyzes in the study, comparisons with a p-value of less than 0.05 were considered statistically significant.

Results

A total of 116 people, 53 healthy and 63 SARS-CoV-2-positive seriously ill patients, were included in this study. Of these, 58.7% were male and 41.3% were female. The age of the evaluated patients was between 23-92 years, and the mean age was calculated as 67.87±14.38 years. Biochemical parameters are shown in Table 1.

In the COVID-19-positive patient group, t copper level of women was found to be higher than that of men. But the difference between the groups was not significant. There is a positive significant relationship between copper level and AST (r=0.36; p=0.01) and LDH (r=0.32; p=0.02) and a significant negative correlation between copper level and T-Bil (r=-0.28; p=0.03).

In the COVID-19 positive patient group, it was found that the zinc level in COVID-19 positive patients did not differ significantly by gender (p>0.05 and Table 1). There was a negative correlation between zinc level and DBil (r=-0.27; p=0.03), CRP (r=-0.26; p=0.04) and procalcitonin (r=-0.37; p=0.01 and Table 1).

Serum Cu and Zn levels from the patient and control groups were compared. It was found that the serum Cu level was significantly lower (patient 68.59±20.7 μg/dL; control 137.0±17.1 μg/dL p<0.0001) and the Zn level was significantly higher (patient 105.8±32.9; control 87.45±87.45; p=0.001) in

COVID-19 patients (Table 1 and Figure 1).

Oxidative stress parameters were studied in COVID-19 patients and control group (Figure 1). TAS and G6PD (glucose-6-phosphate dehydrogenase) values were found to be significantly lower (p=0.0001 and p=0.001, respectively), and TOS and OSI values were found to be significantly higher in patients compared to the control group (p=0.005 and p=0.0005, respectively)

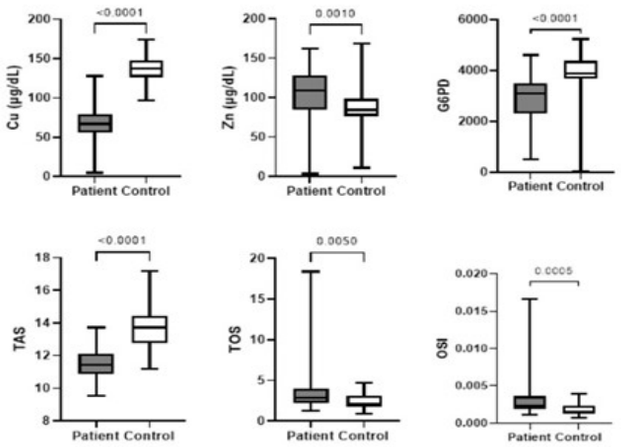

Figure 1. Cu (copper), Zn (zinc), G6PD (glucose-6-phosphate dehydrogenase), TAS (total antioxidant status), TOS (total oxidant status), and OSI (oxidative stress index) levels in COVID-19 patients and healthy controls. Data are expressed as mean ± standard deviation (sd).

Discussion

The inflammatory response plays a critical role in COVID-19, and the inflammatory cytokine storm increases the severity of COVID-19 [9]. Elements such as zinc and copper are well known for their regulatory character in controlling oxidative stress and inflammatory cytokine [10]. Our study showed that serum zinc and copper levels have a relation with infection and inflammation status. Zinc is negatively correlated and copper is positively correlated with inflammation in COVID-19 patients. Our findings in this study support these studies. We found that the Cu level was low and the Zn level was higher in COVID-19 patients compared to the control group consisting of healthy people.

As is known, the barrier function of the immune system acts to prevent pathogens from entering the body from the external environment. Many studies have shown that zinc supplementation improves lung integrity. Disturbances in the integrity of the respiratory epithelium facilitate the entry of COVID-19 as well as co-infecting pathogens and can lead to pathogens entering the bloodstream [11 12]. However, during the acute phase of infection, zinc uptake into cells and urinary excretion of zinc increase, which may lead to a decrease in serum zinc levels [13]. Therefore, with these mentioned mechanisms, zinc plays an important role in the inflammatory response and tends to

Table 1. Value of COVID-19 positive patient biochemistry, hormone, coagulation results, and correlation analysis of copper and zinc

	Mean±sd	Copper		Zinc	
		r	p	r	p
Gender		-0.07	0.59	-0.09	0.49
Glucose (mg/dL)	186.04 ±79.47	-0.01	0.93	0.17	0.18
Urea (mg/dL)	69.09 ± 58.97	-0.05	0.68	-0.02	0.87
Creatinine (mg/dL)	2.38 ± 8.50	0.21	0.10	0.10	0.43
Total Protein (g/L)	20.52 ± 28.64	0.12	0.34	0.07	0.59
ALT (U/L)	48.63 ± 120.53	-0.08	0.52	0.13	0.31
AST (U/L)	41.02 ± 39.33	0.36	0.01*	-0.24	0.06
T Bil (mg/dL)	1.21 ± 3.44	-0.28	0.03*	-0.12	0.34
D Bil (mg/dL)	0.31 ± 0.22	-0.03	0.80	-0.27	0.03*
GGT (IU/L)	79.57 ± 88.49	-0.17	0.19	0.12	0.37
Na (mmol/L)	110.22 ± 49.58	0.04	0.75	0.10	0.43
K (mmol/L)	90.94 ± 64.58	-0.02	0.86	0.04	0.75
Ca (mg/dL)	5.84 ± 2.22	-0.07	0.59	0.01	0.93
P (mg/dL)	3.30 ± 1.35	-0.16	0.27	0.22	0.12
Mg (mg/dL)	2.10 ± 0.37	0.15	0.28	0.16	0.24
LDH (IU/L)	485.62 ± 222.30	0.32	0.02*	-0.20	0.14
CRP (mg/L)	79.96 ± 66.08	0.15	0.26	-0.26	0.04*
Procalcitonin (qg/L)	4.18±15.62	-0.24	0.86	-0.37	0.01*
Ferritin (ng/mL)	843.5±629.5	-0.03	0.81	-0.14	0.26
CK-MB (ng/mL)	4.99±8.27	-0.01	0.91	-0.23	0.09
Troponine (ng/mL)	128.8±265.6	-0.14	0.28	-0.14	0.27
Fibrinogen (g/L)	4.29±1.69	-0.29	0.21	0.14	0.33
D-Dimer (mg/L)	4.06±3.4	0.04	0.72	0.11	0.45
Zinc (µg/dL)	90.71 ± 25.73	-0.07	0.58	-	-
Copper (µg/dL)	278.24 ± 1161.27	-	-	-0.07	0.58

* Statistically significant difference (statistical analysis was done with Spearman Correlation analysis). r : <0.2 Very weak correlation or no correlation. 0.2-0.4 Weak correlation, 0.4-0.6 Moderate correlation, 0.6-08 High correlation, >0.8 is interpreted as a very high correlation.

decrease during the acute phase by increasing zinc uptake into cells. It is seen that the mean zinc level in our patients are close to the lower limit of normal and even lower in men with severe COVID-19. On the other hand, serum zinc level was found to be negatively correlated with D-bil, CRP, and procalcitonin, but positively correlated with D-vit. Recent systematic reviews and meta-analyses of trials with zinc report faster healing from common cold, reduced incidence and prevalence of pneumonia, and reduced mortality when given to severe pneumonia [14 15]. Thus, first results and treatment regimens regarding zinc and D-vit supplementation for COVID-19 risk groups and patients can be anticipated soon [16].

Copper is an essential micronutrient for both pathogens and the patients they infect. There is a progressive increase in serum copper in many cases of infection [17]. When looking at infections in the literature, studies showed that serum copper and urinary copper levels increased in chronic Hepatitis B and HIV patients compared to controls [18]. Our data showed that serum copper level was elevated in severe COVID-19 patients. Serum copper level was found to correlate negatively with total bilirubin and fibrinogen and positively with AST and LDH.

Conclusion

This study showed that serum zinc levels increased, serum copper and G6PD levels increased and decreased in patients diagnosed with COVID-19 compared to controls. In COVID-19 infection, the relationship between zinc, copper, and G6PD changes and oxidative stress index has been demonstrated. These results demonstrated the recently discussed association of zinc with COVID-19 disease. This effect will be better revealed in more comprehensive studies to be planned in the future.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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References

1. Romano M, Ruggiero A, Squeglia F, Maga G, Berisio R. A Structural View of SARS-CoV-2 RNA Replication Machinery: RNA Synthesis, Proofreading and Final Capping. Cells. 2020;9(5):1267.

2. Rani I, Goyal A, Bhatnagar M, Manhas S, Goel P, Pal A, et al. Potential molecular mechanisms of zinc- and copper-mediated antiviral activity of COVID-19. Nutr Res. 2021;92:109-28.

3. te Velthuis AJ, van den Worm SH, Sims AC, Baric RS, Snijder EJ, van Hemert MJ. Zn(2+) inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog. 2010;6(11):e1001176.

4. Read SA, Obeid S, Ahlenstiel C, Ahlenstiel G. The Role of Zinc in Antiviral Immunity. Adv Nutr. 2019;10(4):696-710.

5. Skalny AV, Timashev PS, Aschner M, Aaseth J, Chernova LN, Belyaev VE, et al. Serum Zinc, Copper, and Other Biometals Are Associated with COVID-19 Severity Markers. Metabolites. 2021;11(4):244.

6. Baez-Santos YM, St John SE, Mesecar AD. The SARS-coronavirus papain-like protease: structure, function and inhibition by designed antiviral compounds. Antiviral Res. 2015;115:21-38.

7. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103-11.

8. Karaagac L, Koruk ST, Koruk I, Aksoy N. Decreasing oxidative stress in response to treatment in patients with brucellosis: could it be used to monitor treatment? Int J Infect Dis. 2011;15(5):e346-9.

9. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395(10223):497-506.

10. Aggarwal J, Singh A, Gupta S, Prasad R. Copper and Zinc Status in Psoriasis: Correlation with Severity. Indian J Clin Biochem. 2021;36(1):120-3.

11. Wessels I, Pupke JT, von Trotha KT, Gombert A, Himmelsbach A, Fischer HJ, et al. Zinc supplementation ameliorates lung injury by reducing neutrophil recruitment and activity. Thorax. 2020;75(3):253-61.

12. Bao S, Knoell DL. Zinc modulates cytokine-induced lung epithelial cell barrier permeability. Am J Physiol Lung Cell Mol Physiol. 2006;291(6):L1132-41.

13. Melichar B, Malir F, Tichy M. Urinary zinc excretion in patients with different disorders: the acute phase response in the kidney. Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove. 1993;36(4-5):325-35.

14. Hemila H. Zinc lozenges and the common cold: a meta-analysis comparing zinc acetate and zinc gluconate, and the role of zinc dosage. JRSM Open. 2017;8(5). DOI: 10.1177/2054270417694291.

15. Wang L, Song Y. Efficacy of zinc given as an adjunct to the treatment of severe pneumonia: A meta-analysis of randomized, double-blind and placebo-controlled trials. Clin Respir J. 2018;12(3):857-64.

16. Wessels I, Rolles B, Rink L. The Potential Impact of Zinc Supplementation on COVID-19 Pathogenesis. Front Immunol. 2020;11:1712.

17. Besold AN, Culbertson EM, Culotta VC. The Yin and Yang of copper during infection. J Biol Inorg Chem. 2016;21(2):137-44.

18. Huang Y, Zhang Y, Lin Z, Han M, Cheng H. Altered serum copper homeostasis suggests higher oxidative stress and lower antioxidant capability in patients with chronic hepatitis B. Medicine (Baltimore). 2018;97(24):e11137.

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